



US006372430B1

(12) **United States Patent**
Morrison et al.

(10) **Patent No.: US 6,372,430 B1**
(45) **Date of Patent: Apr. 16, 2002**

(54) **NUCLEIC ACIDS FOR DETECTING ASPERGILLUS SPECIES AND OTHER FILAMENTOUS FUNGI**

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(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 0 days.

(21) Appl. No.: **09/423,233**

(22) PCT Filed: **May 1, 1998**

(86) PCT No.: **PCT/US98/08926**

§ 371 Date: **Jun. 27, 2000**

§ 102(e) Date: **Jun. 27, 2000**

(87) PCT Pub. No.: **WO98/50584**

PCT Pub. Date: **Nov. 12, 1998**

Related U.S. Application Data

(60) Provisional application No. 60/045,400, filed on May 2, 1997.

(51) **Int. Cl.**⁷ **C12Q 1/68**; C07H 21/04; C12P 19/34

(52) **U.S. Cl.** **435/6**; 435/91.1; 536/23.1; 536/24.32; 536/24.3; 536/23.7

(58) **Field of Search** 435/6, 91.1; 536/23.1, 536/24.32, 24.3, 23.7

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(57) **ABSTRACT**

Nucleic acids for detecting *Aspergillus* species and other filamentous fungi are provided. Unique internal transcribed space 2 coding regions permit the development of nucleic acid probes specific for five different species of *Aspergillus*, three species of *Fusarium*, four species of *Mucor*, two species of *Penicillium*, five species of *Rhizopus*, one species of *Rhizomucor*, as well as probes for *Absidia corymbifer*, *Cunninghamella elagans*, *Pseudallescheria boydii*, and *Sporothrix schenckii*. Methods are disclosed for the species-specific detection and diagnosis of infection by *Aspergillus*, *Fusarium*, *Mucor*, *Penicillium*, *Rhizomucor*, *absidia*, *Cunninghamella*, *Pseudallescheria* or *Sporothrix* in a subject. Furthermore, genus-specific probes are also provided for *Aspergillus*, *Fusarium* and *Mucor*, in addition to an all-fungus nucleic acid probe.

29 Claims, No Drawings

NUCLEIC ACIDS FOR DETECTING ASPERGILLUS SPECIES AND OTHER FILAMENTOUS FUNGI

PRIORITY CLAIM

This application claims priority to PCT/US98/08926, filed May 1, 1998, which claims the benefit of U.S. Provisional Application No. 60/045,400, filed May 2, 1997.

This invention was made in the Centers for Disease Control Mycotic Diseases Laboratories, an agency of the United States Government.

TECHNICAL FIELD

This application relates in general to the field of diagnostic microbiology. In particular, the invention relates to the species-specific detection of *Aspergillus*, *Fusarium*, *Mucor*, *Penicillium*, *Rhizopus*, *Rhizomucor*, *Absidia*, *Cunninghamella*, *Pseudallescheria boydii* (*Scedosporium apiospermum*), and *Sporothrix* species.

BACKGROUND OF THE INVENTION

In recent years, chemotherapy for hematological malignancies, and high-dose corticosteroid treatment for organ transplant recipients, along with the spread of AIDS, have greatly increased the number of immunocompromised patients (1, 12, 14, 43). Saprophytic filamentous fungi, such as *Aspergillus*, *Rhizopus*, and *Mucor* species, found in the environment and considered to be of low virulence, are now responsible for an increasing number of infections in the immunocompromised host (17, 20, 43). In addition, these infections are often fulminant and rapidly fatal in immunocompromised patients (7, 11, 12, 20, 44). Morbidity and mortality is extremely high; for example, aspergillosis has a mortality rate of approximately 90% (8, 11).

To complicate matters, diagnosis is difficult and symptoms are often non-specific (18, 27, 29, 42, 44). Antibody-based tests can be unreliable due to the depressed or variable immune responses of immunocompromised patients (2, 9, 18, 46). Antigen detection tests developed to date have fallen short of the desired sensitivity (2, 9, 38). Radiographic evidence can be non-specific and inconclusive (5, 29, 36), although some progress in diagnosis has been made with the advent of computerized tomography (40). However, definitive diagnosis still requires either a positive blood or tissue culture or histopathological confirmation (3, 21). An added complication is that the invasive procedures necessary to obtain biopsy materials are often not recommended in thrombocytopenic patient populations (37, 41).

Even when cultures of blood, lung or rhinocerebral tissues are positive, morphological and biochemical identification of filamentous fungi can require several days for adequate growth and sporulation to occur, delaying targeted drug therapy. Some atypical isolates may never sporulate, making identification even more difficult (23). When histopathology is performed on tissue biopsy sections, the morphological similarities of the various filamentous fungi in tissue make differentiation difficult (16). Fluorescent antibody staining of histopathological tissue sections is not specific unless cross-reactive epitopes are absorbed out which can make the resultant antibody reactions weak (14, 19). Therapeutic choices vary (7, 41, 44) making a test to rapidly and specifically identify filamentous fungi urgently needed for the implementation of appropriately targeted therapy. Early and accurate diagnosis and treatment can decrease morbidity and increase the chances for patient survival (6, 27, 39).

Furthermore, identification of filamentous fungi to at least the species level would be epidemiologically useful (24, 31, 43, 47).

PCR-based methods of detection, which show promise as rapid, sensitive means to diagnose infections, have been used in the identification of DNA from *Candida* species (13, 15, 30) and some other fungi, particularly *Aspergillus* species (31, 33, 45). However, most of these tests are only genus-specific (28, 38) or are directed to detect only single-copy genes (4, 35). Others have designed probes to detect multi-copy genes so as to increase test sensitivity (31, 33) but in doing so have lost test specificity because they have used highly conserved genes, which detect one or a few species but which are also plagued with cross-reactivities to human, fungal or even viral DNA (25, 31, 33).

Therefore, it is an object of the invention to provide improved materials and methods for detecting and differentiating *Aspergillus* and other filamentous fungal species in the clinical and laboratory settings.

SUMMARY OF THE INVENTION

The present invention relates to nucleic acids for detecting *Aspergillus*, *Fusarium*, *Mucor*, *Penicillium*, *Rhizopus*, *Rhizomucor*, *Absidia*, *Cunninghamella*, *Pseudallescheria* (*Scedosporium*), and *Sporothrix* species. Unique internal transcribed spacer 2 coding regions permit the development of probes specific for five different *Aspergillus* species, *A. flavus*, *A. fumigatus*, *A. niger*, *A. terreus*, and *A. nidulans*. The invention thereby provides methods for the species-specific detection and diagnosis of *Aspergillus* infection in a subject. In addition, species probes have been developed for three *Fusarium*, four *Mucor*, two *Penicillium*, five *Rhizopus* and one *Rhizomucor* species, as well as probes for *Absidia corymbifera*, *Cunninghamella elegans*, *Pseudallescheria boydii* (*Scedosporium apiospermum*), and *Sporothrix schenckii*. Generic probes for *Aspergillus*, *Fusarium*, and *Mucor* species have also been developed.

These and other objects, features and advantages of the present invention will become apparent after a review of the following detailed description of the disclosed embodiments and the appended claims.

DETAILED DESCRIPTION OF THE INVENTION

This invention provides a simple, rapid, and useful method for differentiating filamentous fungal species from each other and from other medically important fungi. This invention enables a rapid, simple and useful method to isolate fungal DNA from host samples, and to apply the species- and genus-specific probes for the diagnosis of a disease. Ultimately, these probes can be used for in situ hybridization or in situ PCR diagnostics so that the morphology of host tissue, and microorganisms, remain intact.

The invention provides nucleic acids containing regions of specificity for five *Aspergillus*, three *Fusarium*, four *Mucor*, two *Penicillium*, five *Rhizopus* and one *Rhizomucor* species as well as probes for *Absidia corymbifera*, *Cunninghamella elegans*, *Pseudallescheria boydii* (*Scedosporium apiospermum*), and *Sporothrix schenckii*. These nucleic acids are from the internal transcribed spacer 2 ("ITS2") region of ribosomal deoxyribonucleic acid (rDNA) of the genome of the aforementioned filamentous fungi. The ITS2 region is located between the 5.8S rDNA region and the 28S rDNA region.

In particular, the invention provides nucleic acids from *Aspergillus flavus* (SEQ ID NO:1), *Aspergillus fumigatus*

(SEQ ID NO:2), *Aspergillus niger* (SEQ ID NO:3), *Aspergillus terreus* (SEQ ID NO:4), *Aspergillus nidulans* (SEQ ID NO:5), *Fusarium solani* (SEQ ID NO:6), *Fusarium moniliforme* (SEQ ID NO:7), *Mucor rouxii* (SEQ ID NO:8), *Mucor racemosus* (SEQ ID NO:9), *Mucor plumbeus* (SEQ ID NO:10), *Mucor indicus* (SEQ ID NO:11), *Mucor circinilloides f. circinelloides* (SEQ ID NO:12), *Rhizopus oryzae* (SEQ ID NO:13 and NO:14), *Rhizopus microsporis* (SEQ ID NO:15 and 16), *Rhizopus circinans* (SEQ ID NO:17 and 18), *Rhizopus stolonifer* (SEQ ID NO:19), *Rhizomucor pusillus* (SEQ ID NO:20), *Absidia corymbifera* (SEQ ID NO:21 and 22), *Cunninghamella elegans* (SEQ ID NO:23), *Pseudallescheria boydii* (teleomorph of *Scedosporium apiospermum*) (SEQ ID NO:24, 25, 26, and 27), *Penicillium notatum* (SEQ ID NO:28), and *Sporothrix schenkii* (SEQ ID NO:29). These sequences can be used to identify and distinguish the respective species of *Aspergillus*, *Fusarium*, *Mucor*, *Rhizopus*, and *Penicillium*, and identify and distinguish these species from each other and from *Absidia corymbifera*, *Cunninghamella elegans*, *Pseudallescheria boydii* (*Scedosporium apiospermum*), and *Sporothrix schenkii*.

Furthermore, the invention provides isolated nucleic acid probes derived from GenBank nucleic acid sequences (for *Penicillium marneffei* and *Fusarium oxysporum* only) or from the above nucleic acid sequences which may be used as species-specific identifiers of *Aspergillus flavus* (SEQ ID NO:30 and 31), *Aspergillus fumigatus* (SEQ ID NO:32), *Aspergillus niger* (SEQ ID NO:33), *Aspergillus terreus* (SEQ ID NO:34), *Aspergillus nidulans* (SEQ ID NO:35), *Mucor rouxii* (SEQ ID NO:36), *Mucor plumbeus* (SEQ ID NO:37), *Mucor indicus* (SEQ ID NO:38), *Mucor circinilloides f. circinelloides* (SEQ ID NO:39), *Mucor racemosus* (SEQ ID NO:40), *Rhizopus oryzae* (SEQ ID NO:41), *Rhizopus circinans* (SEQ ID NO:42), *Rhizomucor pusillus* (SEQ ID NO:43), *Rhizopus stolonifer* (SEQ ID NO:44), *Pseudallescheria boydii* (*Scedosporium apiospermum*) (SEQ ID NO:45), *Penicillium notatum* (SEQ ID NO:46), *Penicillium marneffei* (SEQ ID NO:47 and 48), *Fusarium moniliforme* (SEQ ID NO:49), *Fusarium oxysporum* (SEQ ID NO:50), *Fusarium solani* (SEQ ID NO:51), *Cunninghamella elegans* (SEQ ID NO:52, 53, and 54), *Absidia corymbifera* (SEQ ID NO:55), *Sporothrix schenkii* (SEQ ID NO:56), and *Rhizopus microsporus* (SEQ ID NO:57). Such probes can be used to selectively hybridize with samples containing nucleic acids from species of *Aspergillus*, *Fusarium*, *Mucor*, *Rhizopus* (or *Rhizomucor*), *Penicillium*, or from *Absidia corymbifera*, *Cunninghamella elegans*, *Pseudallescheria boydii* (*Scedosporium apiospermum*), and *Sporothrix schenkii*. These fungi can be detected after polymerase chain reaction or ligase chain reaction amplification of fungal DNA and specific probing of amplified DNA with DNA probes labeled with digoxigenin, reacted with anti-digoxigenin antibodies labeled with horseradish peroxidase and a colorimetric substrate, for example. Additional probes can routinely be derived from the sequences given in SEQ ID NOs: 1–29, which are specific for the respective species. Therefore, the probes shown in SEQ ID NOs:30–57 are only provided as examples of the species-specific probes that can be derived from SEQ ID NOs: 1–29.

Generic probes for *Aspergillus* (SEQ ID NO:58), *Fusarium*, (SEQ ID NO:59) and *Mucor* (SEQ ID NO:60) species have also been developed to identify all members of their respective species which are listed above as well as an all-fungus biotinylated probe (SEQ ID NO:61) to capture all species-specific and generic probes listed above for their detection.

By “isolated” is meant nucleic acid free from at least some of the components with which it naturally occurs. By “selective” or “selectively” is meant a sequence which does not hybridize with other nucleic acids to prevent adequate determination of an *Aspergillus*, *Fusarium*, *Mucor*, *Penicillium*, *Rhizopus* or *Rhizomucor* genus or species or of *Absidia corymbifera*, *Cunninghamella elegans*, *Pseudallescheria boydii* (*Scedosporium apiospermum*), or *Sporothrix schenkii* species.

The hybridizing nucleic acid should have at least 70% complementarity with the segment of the nucleic acid to which it hybridizes. As used herein to describe nucleic acids, the term “selectively hybridizes” excludes the occasional randomly hybridizing nucleic acids and thus has the same meaning as “specifically hybridizing”. The selectively hybridizing nucleic acids of the invention can have at least 70%, 80%, 85%, 90%, 95%, 97%, 98%, and 99% complementarity with the segment of the sequence to which it hybridizes.

The invention contemplates sequences, probes and primers which selectively hybridize to the complementary, or opposite, strand of DNA as those specifically provided herein. Specific hybridization with nucleic acid can occur with minor modifications or substitutions in the nucleic acid, so long as functional species-specific or genus-specific hybridization capability is maintained. By “probe” is meant nucleic acid sequences that can be used as probes or primers for selective hybridization with complementary nucleic acid sequences for their detection or amplification, which probes can vary in length from about 5 to 100 nucleotides, or preferably from about 10 to 50 nucleotides, or most preferably about 18 nucleotides. The invention provides isolated nucleic acids that selectively hybridize with the species-specific nucleic acids under stringent conditions and should have at least 5 nucleotides complementary to the sequence of interest. See generally, Maniatis (26).

If used as primers, the invention provides compositions including at least two nucleic acids which hybridize with different regions so as to amplify a desired region. Depending on the length of the probe or primer, target region can range between 70% complementary bases and full complementarity and still hybridize under stringent conditions. For example, for the purpose of diagnosing the presence of the *Aspergillus*, the degree of complementarity between the hybridizing nucleic acid (probe or primer) and the sequence to which it hybridizes (e.g., *Aspergillus* DNA from a sample) is at least enough to distinguish hybridization with a nucleic acid from other yeasts and filamentous fungi. The invention provides examples of nucleic acids unique to each filamentous fungus in the listed sequences so that the degree of complementarity required to distinguish selectively hybridizing from nonselectively hybridizing nucleic acids under stringent conditions can be clearly determined for each nucleic acid.

Alternatively, the nucleic acid probes can be designed to have homology with nucleotide sequences present in more than one species of the fungi listed above. Such a nucleic acid probe can be used to selectively identify a group of species such as the generic probes listed for *Aspergillus* (SEQ ID NO:58), *Fusarium* (SEQ ID NO:59), and *Mucor* (SEQ ID NO:60) as well as all fungi listed (SEQ ID NO:61). Additionally, the invention provides that the nucleic acids can be used to differentiate the filamentous fungi listed in general from other filamentous fungi and yeasts, such as *Candida* species. Such a determination is clinically significant, since therapies for these infections differ.

The invention further provides methods of using the nucleic acids to detect and identify the presence of the

filamentous fungi listed, or particular species thereof. The method involves the steps of obtaining a sample suspected of containing filamentous fungi. The sample may be taken from an individual, such as blood, saliva, lung lavage fluids, vaginal mucosa, tissues, etc., or taken from the environment. The filamentous fungal cells can then be lysed, and the DNA extracted and precipitated. The DNA is preferably amplified using universal primers derived from the internal transcribed spacer regions, 18S, 5.8S and 28S regions of the filamentous fungal rDNA. Examples of such universal primers are shown below as ITS1 (SEQ ID NO:62), ITS3 (SEQ ID NO:63), ITS4 (SEQ ID NO:64). Detection of filamentous fungal DNA is achieved by hybridizing the amplified DNA with a species-specific probe that selectively hybridizes with the DNA. Detection of hybridization is indicative of the presence of the particular genus (for generic probes) or species (for species probes) of filamentous fungus.

Preferably, detection of nucleic acid (e.g. probes or primers) hybridization can be facilitated by the use of detectable moieties. For example, the species-specific or generic probes can be labeled with digoxigenin, and an all-fungus probe, such as described in SEQ ID NO:61, can be labeled with biotin and used in a streptavidin-coated microtiter plate assay. Other detectable moieties include radioactive labeling, enzyme labeling, and fluorescent labeling, for example.

The invention further contemplates a kit containing one or more species-specific probes, which can be used for the detection of particular filamentous fungal species and genera in a sample. Such a kit can also contain the appropriate reagents for hybridizing the probe to the sample and detecting bound probe. The invention may be further demonstrated by the following non-limiting examples.

EXAMPLES

In this example, PCR assay employing universal, fungus-specific primers and a simple, rapid EIA-based format for amplicon detection were used.

Extraction of Filamentous Fungal DNA

A mechanical disruption method was used to obtain DNA from filamentous fungal species and an enzymatic disruption method described previously (13) was used to obtain DNA from yeasts. Filamentous fungi were grown for 4 to 5 days on Sabouraud dextrose agar slants (BBL, division of Becton Dickinson, Cockeysville, Md.) at 35° C. Two slants were then washed by vigorously pipeting 5 mls of 0.01 M potassium phosphate buffered saline (PBS) onto the surface of each slant and the washes were transferred to 500 ml Erlenmeyer flasks containing 250 ml of Sabouraud dextrose broth (BBL). Flasks were then incubated for 4 to 5 days on a rotary shaker (140 rpm) at ambient temperature. Growth was then harvested by vacuum filtration through a sterile Whatman #1 filter paper which had been placed into a sterile Buchner funnel attached to a 2 L side-arm flask. The resultant cellular mat was washed on the filtration apparatus three times with sterile distilled water, removed from the filter paper by gentle scraping with a rubber policeman, and placed into a sterile Petri plate which was then sealed with parafilm and frozen at -20° C. until used.

Just prior to use, a portion of the frozen cellular mat, equal in size to a quarter, was removed and placed into a cold mortar (6" diameter). Liquid nitrogen was added to cover the mat which was then ground into a powder with a pestle. Additional liquid nitrogen was added as needed to keep the mat frozen during grinding.

DNA was then purified using proteinase K and RNase treatment, multiple phenol extractions, and ethanol precipitation by conventional means (26).

PCR amplification

The fungus-specific, universal primer pair ITS3 (5'-GCA TCG ATG AAG AAC GCA GC-3') (SEQ ID NO:63) and ITS4 (5'-TCC TCC GCT TAT TGA TAT GC-3') (SEQ ID NO:64) was used to amplify a portion of the 5.8S rDNA region, the entire ITS2 region, and a portion of the 28S rDNA region for each species as previously described (13, 34). DNA sequencing used this primer pair and also the fungus-specific, universal primer pair ITS1 (5'-TCC GTA GGT GAA CCT GCG G-3') (SEQ ID NO: 62) and ITS4 to amplify a portion of the 18S rDNA region, the entire 5.8S region, the entire ITS1 and ITS2 regions, and a portion of the 28S rDNA region.

A DNA reagent kit (TaKaRa Biomedicals, Shiga, Japan) was used for PCR amplification of genomic DNA. PCR was performed using 2 μ l of test sample in a total PCR reaction volume of 100 μ l consisting of 10 μ l of 10 \times Ex Tag buffer, 2.5 mM each of dATP, dGTP, dCTP, and dTTP, in 8 μ l 0.2 μ M of each primer, and 0.5 U of TaKaRa Ex Tag DNA polymerase. Thirty cycles of amplification were performed in a Perkin-Elmer 9600 thermal cycler (Emeryville, Calif.) after initial denaturation of DNA at 95° C. for 5 minutes. Each cycle consisted of a denaturation step at 95° C. for 30 seconds, an annealing step at 58° C. for 30 seconds, and an extension step at 72° C. for 1 minute. A final extension at 72° C. for 5 minutes followed the last cycle. After amplification, samples were stored at -20° C. until used.

TABLE 1

Synthetic Universal Oligonucleotides Used in PCR and Hybridization Analyses		
Primers or Probes	Nucleotide Sequence (5' to 3')	Chemistry and Location
ITS3	GCA TCG ATG AAG AAC GCA GC (SEQ ID NO:63)	5.85 rDNA universal 5' primer
ITS4	TCC TCC GCT TAT TGA TAT GC (SEQ ID NO:64)	28S rDNA universal 3' primer
ITS1	TCC GTA GGT GAA CCT GCG G (SEQ ID NO:62)	18S rDNA universal 5' primer

DNA Sequencing

Primary DNA amplifications were conducted as described above. The aqueous phase of the primary PCR reaction was purified using QIAquick Spin Columns (Quiagen, Chatsworth, Calif.). DNA was eluted from each column with 50 μ l of heat-sterilized Tris-EDTA buffer (10 mM Tris, 1 mM EDTA, pH 8.0).

Purified DNA was labeled using a dye terminator cycle sequencing kit (ABI PRISM, Perkin Elmer, Foster City, Calif.). One mix was made for each of the primers so that sequencing could be performed in both the forward and reverse directions. The reaction volume (20 μ l) contained 9.5 μ l Terminator Premix, 2 μ l (1 ng) DNA template, 1 μ l primer (3.2 pmol) and 7.5 μ l heat-sterilized distilled H₂O. The mixture was then placed into a pre-heated (96° C.) Perkin Elmer 9600 thermal cycler for 25 cycles of 96° C. for 10 seconds, 50° C. for 5 seconds, 60° C. for 4 minutes. The PCR product was then purified before sequencing using CentriSep spin columns (Princeton Separations, Adelphia, N.J.). DNA was then vacuum dried, resuspended in 6 μ l of formamide-EDTA (5 μ l deionized formamide plus 1 μ l 50 mM EDTA, pH 8.0), and denatured for 2 min at 90° C. prior to sequencing using an automated capillary DNA sequencer (ABI Systems, Model 373, Bethesda, Md.).

The sequencing results were as follows:

Aspergillus flavus 5.8S ribosomal RNA gene, partial sequence, internal transcribed spacer 2, complete sequence, and 28S ribosomal RNA gene, partial sequence.

(SEQ ID NO:1)
 GCTGCCCATC AAGCACGGC TTGTGTGTTG GGTCGTCGTC
 CCCTCTCCGG GGGGGACGGG CCCCAAAGGC AGCGGCGGCA
 CCGCGTCCGA TCCTCGAGCG TATGGGGCTT TGTACCCCGC
 TCTGTAGGCC CGGCCGGCGC TTGCCGAACG CAAATCAATC
 TTTTTCCAGG TTGACCTCGG ATCAGGTAGG GATACCCGCT
 GAACTTCAA

Aspergillus fumigatus 5.8S ribosomal RNA gene, partial sequence, internal transcribed spacer 2, complete sequence, and 28S ribosomal RNA gene, partial sequence.

(SEQ ID NO:2)
 AAACCTTTCAA CAATGGATCT CTTGGTTCCG GCATCGATGA
 AGAACGCAGC GAAATGCGAT AACTAATGTG AATTGCAGAA
 TTCAGTGAAT CATCGAGTCT TTGAACGCAC ATTGCGCCCC
 CTGGTATTCC GGGGGGCATG CCTGTCCGAG CGTCATTGCT
 GCCCATCAAG CACGGCTTGT GTGTTGGGCC CCCGTCCCCC
 TCTCCCGGGG GACGGGCCCG AAAGGCAGCG GCGGCACCGC
 GTCCGGTCCT CGAGCGTATG GGGCTTGTC A CTGCTCTGT
 AGGCCCGGCC GCGCCAGCC GACACCCAAC TTTATTTTTTC
 TAAGGTTGAC CTCGGATCAG GTAGGATAC CCGCTGAACT TAAA

Aspergillus niger 5.8S ribosomal RNA gene, partial sequence, internal transcribed spacer 2, complete sequence, and 28S ribosomal RNA gene, partial sequence.

(SEQ ID NO:3)
 AAACCTTTCAA CAATGGATCT CTTGGTTCCG GCATCGATGA
 AGAACGCAGC GAAATGCGAT AACTAATGTG AATTGCAGAA
 TTCAGTGAAT CATCGAGTCT TTGAACGCAC ATTGCGCCCC
 CTGGTATTCC GGGGGGCATG CCTGTCCGAG CGTCATTGCT
 GCCCTCAAGC ACGGCTTGTG TGTGGGTCG CCGTCCCCCT
 CTCCCGGGG ACGGGCCCGA AAGGCAGCGG CGGCACCGCG
 TCCGATCCTC GAGCGTATGG GGCTTTGTCA CCTGCTCTGT
 AGGCCCGGCC GCGCCTGCC GACGTTATCC AACCATTTTT
 TTCCAGGTTG ACCTCGGATC AGGTAGGGAT ACCCGCTGAA CTAA

Aspergillus terreus 5.8S ribosomal RNA gene, partial sequence, internal transcribed spacer 2, complete sequence, and 28S ribosomal RNA gene, partial sequence.

(SEQ ID NO:4)
 AAACCTTTCAA CAATGGATCT CTTGGTTCCG GCATCGATGA
 AGAACGCAGC GAAATGCGAT AACTAATGTG AATTGCAGAA
 TTCAGTGAAT CATCGAGTCT TTGAACGCAC ATTGCGCCCC

-continued

CTGGTATTCC GGGGGGCAT GCCTGTCCGA GCGTCATTGC
 5 TGCCCTCAAG CCCGGCTTGT GTGTTGGGCC CTCGTCCCCC
 GGCTCCCGGG GGACGGGCC GAAAGGCAGC GCGGCACCG
 CGTCCGGTCC TCGAGCGTAT GGGGCTTCGT CTTCCGCTCC
 10 GTAGGCCCGG CCGGCGCCCG CCGAACGCAT TTATTTGCAA
 CTTGTTTTTT TTTCCAGGTT GACCTCGGAT CAGGT

Aspergillus nidulans 5.8S ribosomal RNA gene, partial sequence, internal transcribed spacer 2, complete sequence, and 28S ribosomal RNA gene, partial sequence.

(SEQ ID NO:5)
 AAACCTTTCAA CAATGGATCT CTTGGTTCCG GCATCGATGA
 20 AGAACGCAGC GAACTGCGAT AAGTAATGTG AATTGCAGAA
 TTCAGTGAAT CATCGAGTCT TTGAACGCAC ATTGCGCCCC
 CTGGCATTCC GGGGGGCATG CCTGTCCGAG CGTCATTGCT
 25 GCCCTCAAGC CCGGCTTGTG TGTGGGTCG TCGTCCCCC
 CCCCAGGGGA CCGGCCCGAA AGGCAGCGC GGCACCGTC
 CGGTCCCTCGA GCGTATGGGG CTTGGTCACC CGCTCGATTA
 30 GGGCCGGCCG GCGCCAGCC GCGTCTCCA ACCTTATCTT
 TCTCAGGTTG ACCTCGGATC AGGTAGGGAT ACCCGCTGAA CTAA

Fusarium solani (strain ATCC62877) internal transcribed spacer 2 and adjacent regions.

(SEQ ID NO:6)
 GAAAATGCGA TAAGTAATGT GAATTGCAGA ATTCAGTGAA
 40 TCATCGAATC TTTGAACGCA CATTGCGCCC GCCAGTATTC
 TGGCGGGCAT GCCTGTTCGA GCGTCATTAC AACCTCAGG
 CCCCCGGGCC TGGCGTTGGG GATCGGCGGA AGCCCCCTGC
 45 GGGCACAACG CCGTCCCCCA AATACAGTGG CCGTCCCGCC
 GCAGCTTCCA TTGCGTAGTA GCTAACACCT CGCAACTGGA
 GAGCGGCGCG GCCACGCCGT AAAACACCCA ACTTCTGAAT
 50 GTTGACCTCG AATCAGGTAG GAATACCCGC TGAACTTAA

Fusarium moniliforme (strain ATCC38519) internal transcribed spacer 2 and adjacent regions.

(SEQ ID NO:7)
 AAATGCGATA AGTAATGTGA ATTGCAAAAT TCAGTGAATC
 ATCGAATCTT TGAACGCACA TTGCGCCCGC CAGTATTCTG
 60 GCGGGCATGC CTGTTGAGC GTCATTTCAA CCCTCAAGCC
 CCCGGGTTTG GTGTTGGGGA TCGGCAAGCC CTTGCGGCAA
 GCCGGCCCCG AAATCTAGTG GCGGTCTCGC TGCAGCTTCC
 65 ATTGCGTAGT AGTAAAACCC TCGCAACTGG TACGCGGCGC

-continued

GGCCAAGCCG TTAAACCCCG AACTTCTGAA TGTTGACCTC
GGATCAGGTA GGAATACCCG CTGAACTTAA

Mucor rouxii (strain ATCC24905) internal transcribed
spacer 2 and adjacent regions.

(SEQ ID NO:8)
AAAGTGCAT AACTAGTGTG AATTGCATAT TCAGTGAATC
ATCGAGTCTT TGAACGCAAC TTGCGCTCAT TGGTATTCCA
ATGAGCACGC CTGTTTCAGT ATCAAAACAA ACCCTCTATC
CAGCATTGTTG TTGAATAGGA ATACTGAGAG TCTCTTGATC
TATTCTGATC TCGAACCTCT TGAAATGTAC AAAGGCCTGA
TCTTGTGTTAA ATGCCTGAAC TTTTTTTTAA TATAAAGAGA
AGCTCTTGCG GTAAACTGTG CTGGGGCCTC CCAAATAATA
CTCTTTTTTAA ATTTGATCTG AAATCAGGCG GGATTACCCG
CTGAACTTAA

Mucor racemosus (strain ATCC22365) internal transcribed
spacer 2 and adjacent regions.

(SEQ ID NO:9)
AAAGTGCAT AACTAGTGTG AATTGCATAT TCAGTGAATC
ATCGAGTCTT TGAACGCAAC TTGCGCTCAT TGGTATTCCA
ATGAGCACGC CTGTTTCAGT ATCAAAACAA ACCCTCTATC
CAACTTTTGT TGTATAGGAT TATTGGGGGC CTCTCGATCT
GTATAGATCT TGAAATCCCT GAAATTTACT AAGGCCTGAA
CTTGTGTTAAA TGCCTGAACT TTTTTTTAAT ATAAAGGAAA
GCTCTTGTA TTAGCTTTGA TGGGGCCTCC CAAATAAATC
TCTTTTAAAT TTGATCTGAA ATCAGGCGGG ATTACCCGCT
GAACTTAA

Mucor plumbeus (strain ATCC4740) internal transcribed
spacer 2 and adjacent regions.

(SEQ ID NO:10)
AAAGTGCAT AACTAGTGTG AATTGCATAT TCAGTGAATC
ATCGAGTCTT TGAACGCAAC TTGCGCTCAT TGGTATTCCA
ATGAGCACGC CTGTTTCAGT ATCAAAACAA ACCCTCTATC
CAACTTTTGT TGTATAGGAT TATTGGGGGC CTCTCGATCT
GTATAGATCT TGAAACCCTT GAAATTTACT AAGGCCTGAA
CTTGTGTTAAT GCCTGAACTT TTTTTTAATA TAAAGGAAAG
CTCTTGTAAT TTAGCTTTGAT GGGGCCTCC AAATAAATCT
TTTTTAAATT TGATCTGAAA TCAGGTGGGA TTACCCGCTG
AACTTAA

Mucor indicus (strain ATCC4857) internal transcribed
spacer 2 and adjacent regions.

(SEQ ID NO:11)
AAAGTGCAT AACTAGTGTG AATTGCATAT TCAGTGAATC
5 ATCGAGTCTT TGAACGCAAC TTGCGCTCAT TGGTATTCCA
TTGAGTACGC CTGTTTCAGT ATCAAAACAA AACCCCTATT
CAAAATTCCT TTTTGAATA GATATGAGTG TAGCAACCTT
10 ACAAGTTGAG ACATTTTAAA TAAAGTCAGG CCATATCGTG
GATTGAGTGC CGATACTTTT TTAATTTTGA AAAGGTAAAG
CATGTTGATG TCCGCTTTTT GGGCCTCCCA AATAACTTTT
15 TAAACTTGAT CTGAAATCAG GTGGGATTAC CCGCTGAACT
TAA

Mucor circinelloides f. circinelloides (strain ATCC1209B)
internal transcribed spacer 2 and adjacent regions.

20 AAAGTGCAT AACTAGTGTG AATTGCATAT TCAGTGAATC
ATCGAGTCTT TGAACGCAAC TTGCGCTCAT TGGTATTCCA
25 ATGAGCACGC CTGTTTCAGT ATCAAAACAA ACCCTCTATC
CAACATTTTT GTTGAATAGG ATGACTGAGA GTCTCTTGAT
CTATTCTGAT CTCGAAAGCTC TTGAAATGTA CAAAGGCCTG
30 ATCTTGTTG AATGCCTGAA CTTTTTTTTA ATATAAAGAG
AAGCTCTTGC GTAAACTGT GCTGGGGCCT CCAAATAAC
ACATCTTTAA ATTTGATCTG AAATCAGGT GGGACTACCC
35 GCTGAACTT AA (SEQ ID NO:12)

Rhizopus oryzae (strain ATCC34965) internal transcribed
spacer 2 and adjacent regions.

40 AGTGCATAA CTAGTGTGAA TTGCATATTC AGTGAATCAT
CGAGTCTTTG AACGCAGCTT GCACTCTATG GTTTTTCTAT
AGAGTACGCC TGCTTCAGTA TCATCACAAA CCCACACATA
45 ACATTTGTTT ATGTGGTGAT GGGTCGCATC GCTGTTTTAT
TACAGTGAGC ACCTAAAATG TGTGTGATTT TCTGTCTGGC
TTGCTAGGCA GGAATATTAC GCTGGTCTCA GGATCTTTTT
50 TTTTGGTTTC CCCAGGAAGT AAAGTACAAG AGTATAATCC
AGTAACTTTC AAATATGAT CTGAAATCAG GTGGGATTAC
CCGCTGAACT TAA (SEQ ID NO:13)

55 *Rhizopus oryzae* (strain ATCC11886) internal transcribed
spacer 2 and adjacent regions.

60 AGTGCATAA CTAGTGTGAA TTGCATATTC AGTGAATCAT
CGAGTCTTTG AACGCAGCTT GCACTCTATG GTTTTTCTAT
AGAGTACGCC TGCTTCAGTA TCATCACAAA CCCACACATA
ACATTTGTTT ATGTGGTAAAT GGGTCGCATC GCTGTTTTAT
65 TACAGTGAGC ACCTAAAATG TGTGTGATTT TCTGTCTGGC

-continued

TTGCTAGGCA GGAATATTAC GCTGGTCTCA GGATCTTTTT
 CTTTGGTTCG CCCAGGAAGT AAAGTACAAG AGTATAATCC
 AGCAACTTTC AAACATGAT CTGAAGTCAG GTGGGATTAC
 CCGCTGAACT TAA (SEQ ID NO:14)

Rhizopus microsporus (strain ATCC14056) internal transcribed spacer 2 and adjacent regions.

AAAGTGCAT AACTAGTGTG AATTGCATAT TCGTGAATCA
 TCGAGTCTTT GAACGCAGCT TGCACCTAT GGATCTTCTA
 TAGAGTACGC TTGCTTCAGT ATCATAACCA ACCCACACAT
 AAAATTTATT TTATGTGGTG ATGGACAAGC TCGGTTAAAT
 TTAATTATTA TACCGATTGT CTAAAATACA GCCTCTTGT
 AATTTTCATT AAATTACGAA CTACCTAGCC ATCGTGCTTT
 TTTGGTCCAA CCAAAAAACA TATAATCTAG GGGTTCTGCT
 AGCCAGCAGA TATTTTAATG ATCTTAACT ATGATCTGAA
 GTCAAGTGGG ACTACCCGCT GAACTTAA (SEQ ID NO:15)

Rhizopus microsporus (strain ATCC12276) internal transcribed spacer 2 and adjacent regions.

AAAGTGCAT AACTAGTGTG AATTGCATAT TCGTGAATCA
 TCGAGTCTTT GAACGCAGCT TGCACCTAT GGATCTTCTA
 TAGAGTACGC TTGCTTCAGT ATCATAACCA ACCCACACAT
 AAAATTTATT TTATGTGGTG ATGGACAAGC TCGGTTAAAT
 TTAATTATTA TACCGATTGT CTAAAATACA GCCTCTTGT
 AATTTTCATT AAATTACGAA CTACCTAGCC ATCGTGCTTT
 TTTGGTCCAA CCAAAAAACA TATAATCTAG GGGTTCTGCT
 AGCCAGCAA TATTTTAATG ATCTTAACT TATGATCTGA
 AGTCAAGTGG GACTACCCGC TGAACCTAA (SEQ ID NO:16)

Rhizopus circinans (strain ATCC34106) internal transcribed spacer 2 and adjacent regions.

AAATTGCGAT AACTAGTGTG AATTGCATTT TCAGTGAATC
 ATCGAGTCTT TGAACGCAT CTGCGCTCT TGGGATTCTT
 CCCTAGAGCA CACTTGCTTC AGTATCATAA CAAAACCCCTC
 ACCTAATATT TTTTTTTTTT AAAAAAAAAA TATTAGAGTG
 GTATTGGGGT CTCTTTGGTA ATTCTTTGTA ATTATAAAAG
 TACCCTTAAA TGTCATAAAC AGGTTAGCTT TAGCTTGCCCT
 TTAAAGATCT TCTTAGGGTA TCATTACTTT TCGTAAATCT
 TTAATAGGCC TGTCACATAA TTCTACCCTT AAATTTCTTA
 AACCTTGATC TGAAGTCAAG TGGGAGTACC CGCTGAACTT AA
 (SEQ ID NO:17)

Rhizopus circinans (strain ATCC34101) internal transcribed spacer 2 and adjacent regions.

AAATTGCGAT AACTAGTGTG AATTGCATTT TCAGTGAATC
 ATCGAGTCTT TGAACGCATC TTGCGCTCTT GGGATTCTTC
 CCTAGAGCAC ACTTGCTTCA GTATCATAAC AAAACCCCTCA
 CCTAATATTT TTTTTTAAAA AAAAAAATA TTAGAGTGGT
 ATTGGGGTCT CTTTGGTAAT TCTTTGTAAT TATAAAAGTA
 CCCTTAAATG TCATAAACAG GTTAGCTTTA GCTTGCCTTT
 AAAGATCTTC TTAGGGTATC ATTACTTTTC GTAAATCTTT
 AATAGGCCCTG TCACATAAAT CTACCCTTAA ATTTCTTAAA
 CCTTGATCTG AAGTCAAGTG GGAGTACCCG CTGAACTTAA
 (SEQ ID NO:18)

Rhizopus stolonifer (strains ATCC14037 and 6227A) internal transcribed spacer 2 and adjacent regions.

AAAGTGCAT AACTAGTGTG AATTGCATAT TCAGTGAATC
 ATCGAGTCTT TGAACGCAAC TTGCACTCTA TGGTTTTCCG
 TAAAGTACGC TTGCTTCAGT ATCATAAAGA CCCCATCCTG
 ATTATTATTT TTTTATTAAA ATAATTAATT TTGGAGATAA
 TAAAAATGAG GCTCTTCTT TTCTTTTTTT TTTTTTTAAA
 AAAAAGGGGG GGAAAGGGTC TTTTAAAATG GGCAAATCT
 GGGTTTTTTTA CTAAACCTGA ACTCCCCCA AAAATTCAA
 AAAAAAAAAA TGGGTTTTAC CAAATTTTTT TTTTTTTTCT
 CCTTTTTGTG TAGTTAATAC TCTATTAAAT TTATTTACTT
 GGTATTATAA CGATTATGCA AGAAGGGAGA GAACAAAGAA
 TAATGAAAGA GAGTTTTTAA ATAAATCTT TTTTCATTTT
 TTCAATCAAT GATCTGAAGT CAAGTGGGAT TACCCGCTGA
 ACTTAA (SEQ ID NO:19)

Rhizomucor pusillus (strain ATCC36606) internal transcribed spacer 2 and adjacent regions.

AAATTGCGAA AAGTAATGCG ATCTGCAGCC TTTGCGAATC
 ATCGAATTCT CGAACGCACC TTGCACCCTT TGGTTCATCC
 ATTGGGTACG TCTAGTTCAG TATCTTTATT AACCCCTAAA
 GGTTTATTTT TTGATAAATC TTTGGATTG CGGTGCCTGAT
 GGATTTTCAT CCGTTCAAGC TACCCGAACA ATTTGTATGT
 TGTTGACCCT TGATATTTCC TTGAGGGCTT GCATTGGTAT
 CTAATTTTTT ACCAGTGTGC TTCGAGATGA TCAAGTATAA
 AGGTCAATCA ACCACAAATA AATTTCAACT ATGGATCTGA
 ACTTAGATGG GATTACCCGC TGAACCTAA (SEQ ID NO:20)

Absidia corymbifera (strain ATCC46774) internal transcribed spacer 2 and adjacent regions.

AAAGTGCAT AATTATTGCG ACTTGCAATC ATAGCGAATC
 ATCGAGTTCT CGAACGCATC TTGCGCCTAG TAGTCAATCT
 ACTAGGCACA GTTGTTCAG TATCTGCAAC TACCAATCAG
 TTCAACTTGG TTCTTTGAAC CTAAGCGAGC TGGAAATGGG
 CTTGTGTTGA TGGCATTGAG TTGCTGTCAT GGCCTTAAAT
 ACATTTAGTC CTAGGCAATT GGCTTTAGTC ATTTGCCGGA
 TGAGACTCT AGAGTGCCCTG AGGAGCAACG ACTTGTTAG
 TGAGTTCATA ATTCCAAGTC AATCAGTCTC TTCTTGAAC
 AGGTCTTAAT CTTTATGGAC TAGTGAGAGG ATCTAACTTG
 GGTCTTCTCT TAAAACAAAC TCACATCTAG ATCTGAAATC
 AACTGAGATC ACCCGCTGAA CTAA (SEQ ID NO:21)

Absidia corymbifera (strain ATCC46773) internal transcribed spacer 2 and adjacent regions.

AAAGTGCAT AATTATTGCG ACTTGCAATC ATAGTGAATC
 ATCGAGTTCT TGAACGCATC TTGCGCCTAG TAGTCAATCT
 ACTAGGCACA GTTGTTCAG TATCTGCATC CACCAATCAA
 CTTAACCTTT TGTGTTGAGT TGGAACGGG CTTCTAGTTG
 ATGGCATTTA GTTGCTGTCA TGGCCTTAAA TCAATGTCCCT
 AGGTGTTAGA ACATCTAACA CCGGATGGAA ACTTTAGAGC
 GCTTTAAGAG CAGCTTGGTT AGTGAGTTCA ATAATTCCAA
 GCATTAAGTC TTTTAATGAA CTAGCTTTTC TATCTATGGG
 ACACTACTTG GAGAAATCCA AGTAACCTTT AACTCCCAT
 TTAGATCTGA AATCAACTGA GACCACCCGC TGAACCTAA
 (SEQ ID NO:22)

Cunninghamella elegans (strain ATCC42113) internal transcribed spacer 2 and adjacent regions.

AAATCGCGAT ATGTAATGTG ACTGCCTATA GTGAATCATC
 AAATCTTTGA AACGCATCTT GCACCTTATG GTATTCCATA
 AGGTACGTCT GTTTCAGTAC CACTAATAAA TCTCTCTCTA
 TCCTTGATGA TAGAAAAAAA AAAAATAATT TTTACTGGGC
 CCGGGGAATC CTTTTTTTTT TTTAATAAAA AGGACCAATT
 TTGGCCAAA AAAAAGGGT GAACTTTTTT TACCAGATCT
 TGCATCTAGT AAAAACCTAG TCGGCTTTAA TAGATTTTTA
 TTTTCTATTA AGTTTATAGC CATTCTTATA TTTTTTAAAA
 TCTTGGCCTG AAATCAGATG GGATACCCGC TGAACCTAA
 (SEQ ID NO:23)

Pseudallescheria boydii (strain ATCC44328) internal transcribed spacer 2 and adjacent regions (teleomorph of *Scedosporium apiospermum*).

AAATGCGATA AGTAATGTAA ATTGCAAAAT TCAGTGAATC
 ATCGAATCTT TGAAACGCAC ATTGCGCCCG GCAGTAATCT
 GCCGGGCATG CCTGTCCGAG CGTCATTTCA ACCCTCGAAC
 CTCCGTTTC CTAGGGGAAAG CCTAGGGTTCG GTGTTGGGGC
 GCTACGGCAA GTCTTCGCAA CCCCCGTAGG CCCTGAAATA
 CAGTGGCGGT CCCGCCGCGG TTGCCTTCTG CGTAGTAAGT
 CTCTTTTGCA AGCTCGCATT GGGTCCCAGC GGAGGCCTGC
 CGTCAAACCA CCTAACAACT CCAGATGGTT TGACCTCGGA
 TCAGGTAGGG TTACCCGCTG AACTTAA (SEQ ID NO:24)

Pseudallescheria boydii (strain ATCC36282) internal transcribed spacer 2 and adjacent regions (teleomorph of *Scedosporium apiospermum*).

GAAATGCGAT AAGTAATGTG AATTGCAGAA TTCAGTGAAT
 CATCGAATCT TTGAAACGCA CATTGCGCCC GGCAGTAATC
 TGCCGGGCAT GCCTGTCCGA GCGTCATTTT AACCTCGAA
 CCTCCGTTTC CTCAGGGAAG CTCAGGGTTCG GTGTTGGGGC
 GCTACGGCAA GTCTTCGCAA CCCTCCGTAG GCCCTGAAAT
 ACAGTGGCGG TCCCAGCGCG GTTGCCTTCT GCGTAGAAGT
 CTCTTTTGCA AGCTCGCATT GGGTCCCAGC GGAGGCCTGC
 CGTCAAACCA CCTATAACTC CAAATGGTTT GACCTCGGAT
 CAGGTAGGGT TACCCGCTGA AACTTAA (SEQ ID NO:25)

Scedosporium apiospermum (strain ATCC64215) internal transcribed spacer 2 and adjacent regions.

GAAATGCGAT AAGTAATGTG AATTGCAGAA TTCAGTGAATC
 ATCGAATCTT TGAACGCACA TTGCGCCCGG CAGTAATCTG
 CCGGGCATGC CTGTCCGAGC GTCATTTCAA CCCTCGAACC
 TCCGTTTCCT CAGGGAAGCT CAGGGTCGGT GTTGGGGCGC
 TACGGCGAGT CTTCGCGACC CTCCGTAGGC CCTGAAATAC
 AGTGGCGGTC CCGCCGCGGT TGCCTTCTGC GTAGTAAGTC
 TCTTTTGCAA GCTCGCATTG GGTCCCAGC GAGGCCTGCC
 GTCAAACCAC CTATAACTCC AGATGGTTTG ACCTCGGATC
 AGGTAGGTAC CCGCTGAACT TAA (SEQ ID NO:26)

Scedosporium apiospermum (strain ATCC46173) internal transcribed spacer 2 and adjacent regions.

AAATGCGATA AGTAATGTGA ATTGCAGAAT TCAGTGAATC
 ATCGAATCTT TGAACGCACA TTGCGCCCGG CAGTAATCTG
 CCGGGCATGC CTGTCCGAGC GTCATTTCAA CCCTCGAACC
 TCCGTTTCCT CAGGGAAGCT CAGGGTCGGT GTTGGGGCGC
 TACGGCGAGT CTTCGCGACC CTCCGTAGGC CCTGAAATAC

-continued

AGTGGCGGTC CCGCCGCGGT TGCCTTCTGC GTAGTAAGTC
 TCTTTTGCAA GCTCGCATTG GGTCCCGGCG GAGGCCTGCC
 GTCAAACCAC CTATAACTCC AGATGGTTTG ACCTCGGATC
 AGGTAGGTAC CCGCTGAACT TAA (SEQ ID NO:27)

Penicillium notatum (strain ATCC10108) internal transcribed spacer 2 and adjacent regions.

AAATGCGATA CGTAATGTGA ATTGCAAATT CAGTGAATCA
 TCGAGTCTT TGAACGCACA TTGCGCCCCC TGGTATTCCG
 GGGGGCATGC CTGTCCGAGC GTCATTGCTG CCCTCAAGCA
 CGGCTTGTGT GTTGGGCCCC GTCCTCCGAT CCCGGGGGAC
 GGGCCCCAAA GGCAGCGGCG GCACCGCGTC CGGTCCCTCGA
 GCGTATGGGG CTTTGTCAAC CGCTCTGTAG GCCCCGCCGG
 CGCTTGCCGA TCAACCCAAA TTTTATCCA GGTGACCTC
 GGATCAGGTA GGGATACCCG CTGAACTTAA (SEQ ID NO:28)

Sporothrix schenckii (strain ATCC14284) internal transcribed spacer 2 and adjacent regions.

GAAATGCGAT ACTAATGTGA ATTGCAGAAT TCAGCGAACC
 ATCGAATCTT TGAACGCACA TTGCGCCCGC CAGCATTCTG
 GCGGGCATGC CTGTCCGAGC GTCATTTCCC CCCTCACGCG
 CCCCCTTGCG CGCTGGTGTG GGGGCGCCCT CCGCTGGCG
 GGGGGCCCC GAAAGCGAGT GGCGGGCCCT GTGGAAGGCT
 CCGAGCGCAG TACCGAACGC ATGTTCTCCC CTCGCTCCGG
 AGGCCCCCA GGCGCCCTGC CCGTGAAAAC GCGCATGACG
 CGCAGCTCTT TTTACAAGGT TGACCTCGGA TCAGGTGAGG 2
 ATACCCGCTG ACTTAA (SEQ ID NO:29)

Contamination Precautions

Precautions were taken to avoid possible contamination of PCR samples by following the guidelines of Fujita and Kwok (13, 22). All buffers and distilled water used for PCR assays were autoclaved and fresh PCR reagents were aliquoted prior to use. Physical separation of laboratory areas used to prepare PCR assays and to analyze PCR products, and the use of aerosol-resistant pipette tips, reduced possible cross-contamination of samples by aerosols. Appropriate negative controls were included in each test run, including controls omitting either the primer or the DNA template during PCR assays.

Agarose gel Electrophoresis

Gel electrophoresis was conducted in TBE buffer (0.1 M Tris, 0.09 M boric acid, 1 mM EDTA, pH 8.4) at 80 V for 1 to 2 hours using gels composed of 1% (w/vol) agarose (International Technologies, New Haven, Conn.) and 1%

(w/vol) NuSieve agar (FMC Bioproducts, Rockland, Me.). Gels were stained with 0.5 μ g of ethidium bromide (EtBr) per ml of distilled H₂O for 10 minutes followed by three serial washes for 10 minutes each with distilled H₂O.

Microtitration Plate Enzyme Immunoassay for the Detection of PCR Products

Amplicons were detected using species-specific and genus probes labeled with digoxigenin and an all-filamentous fungal probe labeled with biotin in a streptavidin-coated microtiter plate format (13, 34). Ten μ l of PCR product was added to each 1.5 ml Eppendorf tube. Single-stranded DNA was then prepared by heating the tubes at 95° C. for 5 minutes and cooling immediately on ice. Two-tenths of a ml of hybridization solution [4 \times SSC (saline sodium citrate buffer, 0.6 M NaCl, 0.06 M trisodium citrate, pH 7.0) containing 20 mM Hepes, 2 mM EDTA, and 0.15% (vol/vol) Tween 20] supplemented with 50 ng/ml each of the all-*Aspergillus* biotinylated probe and a species-specific digoxigenin-labeled probe was added to each tube containing denatured PCR product. Tubes were mixed by inversion and placed in a water bath at 37° C. to allow probes to anneal to PCR product DNA. After 1 hour, 100 μ l of each sample was added to duplicate wells of a commercially prepared streptavidin-coated microtitration plate (Boehringer Mannheim, Indianapolis, Ind.). The plate was incubated at ambient temperature for 1 hour with shaking, using a microtitration plate shaker (manufactured for Dynatech by CLTI, Middletown, N.Y.). Plates were washed 6 times with 0.01 M potassium phosphate buffered saline, pH 7.2, containing 0.05% Tween 20 (PBST). Each well then received 100 μ l of horseradish peroxidase-conjugated, anti-digoxigenin Fab fragment (Boehringer Mannheim) diluted 1:1000 in hybridization buffer. After incubation at ambient temperature for 30 minutes with shaking, the plate was washed 6 times with PBST. One hundred μ l of a mixture of one volume of 3, 3', 5, 5'-tetramethyl benzidine peroxidase substrate (Kirkegaard and Perry Laboratories, Inc., Gaithersburg, Md.) and one volume of peroxidase solution (Kirkegaard and Perry Laboratories) was added to each well and the plate was placed at ambient temperature for 10 minutes for color development. The A₆₅₀ nm of each well was determined with a microtitration plate reader (UV Max, Molecular Devices, Inc., Menlo Park, Calif.). The absorbance value for the reagent blank, where DNA was absent but replaced with distilled H₂O, was subtracted from each test sample.

Statistical Analysis

The Student's t test was used to determine differences between sample means. Means are expressed as the mean plus or minus the standard error from the mean. Differences were considered significant when P<0.05.

The following probes were used to detect and distinguish each species.

TABLE 2

PROBES	Probe Sequences	
	5' to 3' OLIGONUCLEOTIDE SEQUENCE	
<u>Generic Biotin Probe</u>	5' end-labeled biontynylated	
	probe	
	5.8S region of rDNA	
B-58	GAA TCA TCG A(AG)T CTT TGA ACG	SEQ ID NO 61
Digoxigenin-probe	5' end-labeled digoxigenin probe	
	ITS2 region of rDNA	
<u>Aspergillus species</u>		
<i>A. flavus</i> 22	GCA AAT CAA TCT TTT TCC	SEQ ID NO 30
<i>A. flavus</i> 23	GAA CGC AAA TCA ATC TTT	SEQ ID NO 31
<i>A. fumigatus</i>	CCG ACA CCC ATC TTT ATT	SEQ ID NO 32
<i>A. niger</i>	GAC GTT ATC CAA CCA TTT	SEQ ID NO 33
<i>A. nidulans</i>	GGC GTC TCC AAC CTT ATC	SEQ ID NO 35
<i>A. terreus</i>	GCA TTT ATT TGC AAC TTG	SEQ ID NO 34
<u>Fusarium species</u>		
<i>F. moniliforme</i>	TCT AGT GAC GGT CTC GCT	SEQ ID NO 49
<i>F. oxysporum</i>	CGT TAA TTC GCG TTC CTC	SEQ ID NO 50
<i>F. solani</i>	CTA ACA CCT CGC AAC TGG AGA	SEQ ID NO 51
<u>Mucor species</u>		
<i>M. circinelloides</i>	AAC ATT TTT GTG AAT AGG ATG	SEQ ID NO 39
<i>M. indicus</i>	CGT GGA TTG AGT GCC GAT	SEQ ID NO 38
<i>M. plumbeus</i>	GAA ACC CTT GAA ATT	SEQ ID NO 37
<i>M. rouxii</i>	GAA TAG GAA TAC TGA GAG	SEQ ID NO 36
<i>M. racemosus</i>	GAA ATC CCT GAA ATT	SEQ ID NO 40
<u>Penicillium species</u>		
<i>Penicillium marneffeii</i> 1	GGG TTG GTC ACC ACC ATA	SEQ ID NO 47
<i>Penicillium marneffeii</i> 2	TGG TCA CCA CCA TAT TTA	SEQ ID NO 48
<i>Penicillium notatum</i>	GAT CAA CCC AAA TTT TTA	SEQ ID NO 46
<u>Rhizopus species</u>		
<i>R. circinans</i>	CTT AGG GTA TCA TTA CTT	SEQ ID NO 42
<i>R. microsporus</i>	CAT ATA ATC TAG GGG TTC	SEQ ID NO 57
<i>R. oryzae</i>	GAG TAT AAT CCA G(CT)A ACT	SEQ ID NO 41
<i>R. stolonifer</i>	CTT GGT ATT ATA ACG ATT	SEQ ID NO 44
<i>Rhizomucor pusillus</i>	TCC TTG AGG GCT TGC ATT	SEQ ID NO 43
<u>Other Genera</u>		
<i>Absidia corymbifera</i>	GTT GCT GTC ATG GCC TTA	SEQ ID NO 55
<i>Cunninghamella elegans</i> 4	TAG TCG GCT TTA ATA GAT	SEQ ID NO 52
<i>Cunninghamella elegans</i> 5	TAT TAA GTT TAT AGC CAT	SEQ ID NO 53
<i>Cunninghamella elegans</i> 6	TAA GTT TAT AGC CAT TCT	SEQ ID NO 54
<i>Pseudallescheria boydii</i>	AAG TCT CTT TTG CAA GCT	SEQ ID NO 45
<i>Sporothrix schoenckii</i>	GAC GCG CAG CTC TTT TTA	SEQ ID NO 56
<u>Genus Probes</u>		
G-ASPERGILLUS	CCT CGA GCG TAT GGG GCT	SEQ ID NO 58
G-FUSARIUM	CCC AAC TTC TGA ATG TTG	SEQ ID NO 59
G-MUCOR	(AC)TG GGG CCT CCC AAA TAA	SEQ ID NO 60

Species-specific probes to the ITS2 region of rDNA for *Aspergillus fumigatus* (SEQ ID NO:32), *A. flavus* (SEQ ID NO:31), *A. niger* (SEQ ID NO:33), *A. terreus* (SEQ ID NO:34), and *A. nidulans* (SEQ ID NO:35) correctly identified each of the respective species ($P < 0.001$), and gave no

false-positive reactions with *Rhizopus*, *Mucor*, *Fusarium*, *Penicillium*, or *Candida* species. The *A. flavus* probe also recognized *A. oryzae*, which belongs to the *A. flavus* group. Identification time was reduced from a mean of 5 days by conventional methods to 8 hours.

TABLE 3

Aspergillus Probes					
Fungus	<i>A. fumigatus</i>	<i>A. nidulans</i>	<i>A. niger</i>	<i>A. terreus</i>	<i>A. flavus</i>
<i>A. fumigatus</i> (n = 6)	2.197 ± 0.187	0.002	0.000	0.001	0.001
<i>A. nidulans</i> (n = 3)	0.001	1.315 ± 0.464	0.002	0.000	0.001
<i>A. niger</i> (n = 5)	0.000	0.000	1.242 ± 0.471	0.001	0.003
<i>A. terreus</i> (n = 4)	0.001	0.000	0.001	1.603 ± 0.378	0.001
<i>A. flavus</i> (n = 6)	0.001	0.001	0.000	0.001	2.043 ± 0.390
<i>A. oryzae</i> (n = 2)	0.001	0.002	0.001	0.001	2.445 ± 0.106
<i>A. parasitica</i> (n = 1)	0.001	0.002	0.002	0.002	0.051
<i>A. clavus</i> (n = 1)	0.005	0.005	0.006	0.005	0.003
<i>C. albicans</i> (n = 1)	0.002	0.001	0.002	0.000	0.000
<i>C. parasitosis</i> (n = 1)	0.001	0.002	0.002	0.002	0.001
<i>C. glabrata</i> (n = 1)	0.001	0.003	0.001	0.001	0.005
<i>C. krusei</i> (n = 1)	0.002	0.002	0.002	0.001	0.001
<i>C. tropicalis</i> (n = 1)	0.002	0.002	0.001	0.000	0.001
<i>F. moniliforme</i> (n = 1)	0.003	0.003	0.001	0.001	0.001
<i>F. solani</i> (n = 1)	0.006	0.002	0.001	0.000	0.001
<i>R. oryzae</i> (n = 1)	0.001	0.001	0.001	0.001	0.001
<i>M. racemosus</i> (n = 1)	0.001	0.002	0.005	0.002	0.000
<i>P. notatum</i> (n = 1)	0.001	0.002	0.002	0.002	0.000
Avg ± SD	0.001 ±	0.001 ±	0.000 ±	0.000 ±	0.002 ±
negative controls	0.002	0.001	0.002	0.002	0.010

Species-specific probes to the ITS2 region of rDNA for *Fusarium oxysporum*, *F. solani*, and *F. moniliforme*, correctly identified each of the respective species (P<0.001), and gave no false-positive reactions with Blastomyces,

⁴⁰ Apophysomyces, Candida, Aspergillus, Mucor, Penicillium, Rhizopus, Rhizomucor, Absidia, Cunninghamella, Pseudallescheria, Sporothrix, or Neosartorya. Empty boxes in Table 4 represent zero probe reactivity.

TABLE 4

Fusarium Probes				
Fungus	<i>F. oxysporum</i>	<i>F. solani</i>	<i>F. moniliforme</i>	Generic Fusarium
<i>F. oxysporum</i> (n = 3)	1.40 ± 0.13			1.76 ± 0.27
<i>F. solani</i> (n = 5)		1.57 ± 0.07		1.35 ± 0.28
<i>F. moniliforme</i> (n = 2)	0.01	0.91	1.40 ±	1.34 ±
Negative control				
<i>A. fumigatus</i>				
<i>A. flavus</i>				
<i>A. niger</i>				
<i>A. nidulans</i>				
<i>A. terreus</i>				
<i>A. parasiticus</i>				
<i>A. clavatus</i>				
<i>P. marneffei</i>		0.01	0.01	
<i>P. notatum</i>	0.01	0.01	0.01	
<i>Rhizopus oryzae</i>		0.03	0.01	
<i>Rhizopus microsporus</i>		0.01	0.01	

TABLE 4-continued

Fungus	Fusarium Probes			Generic Fusarium
	<i>F. oxysporum</i>	<i>F. solani</i>	<i>F. moniliforme</i>	
<i>Rhizopus circinans</i>		0.01	0.01	
<i>Rhizopus stolonifer</i>	0.01	0.01		
<i>Rhizomucor pusillus</i>	0.03	0.02		
<i>M. racemosus</i>				
<i>M. circinelloides</i>				
<i>M. rouxii</i>				
<i>M. plumbeus</i>				
<i>M. indicus</i>				
<i>Absidia corymbifera</i>		0.01	0.01	
<i>Cunninghamella elegans</i>		0.01	0.02	
<i>P. boydii</i>	0.02			
<i>Sporothrix schenckii</i>		0.01	0.01	
<i>C. albicans</i>				
<i>C. tropicalis</i>				
<i>C. krusei</i>				
<i>C. parasitosis</i>				
<i>C. glabrata</i>				
<i>Neosartorya fischeri</i>		0.01		
<i>Blastomyces dermatitidis</i>				
<i>Apophysomyces elegans</i>				
Average of negative controls	0.001 ± 0.002	0.005 ± 0.01	0.004 ± 0.006	

Species-specific probes to various other zygomycetes are presented in Table 5, showing correct identification of each species and no false positives. The exceptions are that the *M. circinelloides* probe hybridized with the *M. rouxii* DNA and the *M. plumbeus* probe hybridized with the *M. racemosus*

DNA. However, the *M. rouxii* probe did not hybridize with *M. circinelloides* DNA, nor did the *M. racemosus* probe hybridize with *M. plumbeus* DNA. Therefore, by a process of elimination, each species can be correctly identified. Empty boxes in Table 5 represent zero probe reactivity.

TABLE 5

FUNGUS	Zygomycetes Probes											
	D-probes RORY	RMIC	RCIR	RSTOL	RPUS	MRACE	MCIR	MRX	MPLUM	MIND	ABS	CUN
<i>R. oryzae</i> (n = 5)	1.50 ± 0.48				0.01							
<i>R. microsporus</i> (n = 5)		0.96 ± 0.61										
<i>R. circinans</i> (n = 3)			1.56 ± 0.19									
<i>R. stolonifer</i> (n = 5)				2.53 ± 0.07			0.01					
<i>Rhizomucor pusillus</i> (n = 2)					1.10 ± 0.68							
<i>M. racemosus</i> (n = 6)				0.01		2.02 ± 0.34				0.29 ± 0.52		
<i>M. circinelloides</i> (n = 3)							1.63 ± 0.37	0.01	0.02			
<i>M. rouxii</i> (n = 1)							1.77	0.76				
<i>M. plumbeus</i> (n = 2)									2.14 ± 0.25			
<i>M. indicus</i> (n = 1)		0.01								1.70 ± 0.04		
<i>Absidia corymbifera</i> (n = 2)					0.01				0.01		1.61 ± 0.08	
<i>Cunninghamella elegans</i> (n = 2)		0.01										2.26 ± 0.03
<u>Negative control</u>												
<i>A. fumigatus</i>									0.01	0.02		
<i>A. flavus</i>					0.01					0.05		
<i>A. niger</i>								0.01				
<i>A. nidulans</i>									0.01	0.01		
<i>A. terreus</i>	0.01											
<i>A. parasiticus</i>					0.01					0.03		

TABLE 5-continued

FUNGUS	Zygomycetes Probes											
	D-probes RORY	RMIC	RCIR	RSTOL	RPUS	MRACE	MCIR	MRX	MPLUM	MIND	ABS	CUN
<i>A. clavatus</i>										0.02		
<i>P. marneffei</i>			0.01									
<i>P. notatum</i>										0.03		
<i>F. oxysporum</i>								0.01				
<i>F. solani</i>												
<i>F. moniliforme</i>	0.01			0.01				0.01		0.01		
<i>P. boydii</i>	0.02											
<i>Sporothrix schenckii</i>												
<i>C. albicans</i>												
<i>C. tropicalis</i>												
<i>C. krusei</i>												
<i>C. parasilosis</i>												
<i>C. glabrata</i>												
<i>Neosartorya fischeri</i>			0.01									
<i>Blastomyces dermatitidis</i>												
<i>Apophysomyces elegans</i>												
Average	0.001 ± .004	0.001 ± 0.02	0.000 ± 0.002	0.000 ± 0.003	0.001 ± 0.003	0.001 ± 0.002	0.001 ± 0.002	0.001 ± 0.003	0.003 ± 0.005	0.005 ± 0.01	0.001 ± 0.001	

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Species-specific probes to various other fungi are presented in Table 6, showing correct identification of each

species and no false positives. Empty boxes in Table 6 represent zero probe reactivity.

TABLE 6

Pseudallescheria and Sporothrix Probes				
Fungus	<i>P. boydii</i>	<i>P. marneffei</i>	<i>P. notatum</i>	<i>Sporothrix schenckii</i>
<i>P. boydii</i> (n = 4)	1.65 ± 0.48			
<i>P. marneffei</i> (n = 3)	0.01	1.24 ± 0.12		
<i>P. notatum</i> (n = 3)			1.93 ± 0.25	
<i>Sporothrix schenckii</i> (n = 3)	0.01			1.94 ± 0.25
Negative control				
<i>A. fumigatus</i>	0.01			
<i>A. flavus</i>				
<i>A. niger</i>				
<i>A. nidulans</i>				
<i>A. terreus</i>				
<i>A. parasiticus</i>				
<i>A. clavatus</i>			0.11	
<i>F. oxysporum</i>		0.10		
<i>F. solani</i>		0.14		
<i>F. moniliforme</i>		0.08		
<i>R. oryzae</i>	0.01			
<i>R. microsporus</i>	0.01			
<i>R. circinans</i>	0.01			
<i>R. stolonifer</i>	0.01			
<i>Rhizomucor pusilus</i>				
<i>M. racemosus</i>		0.04		
<i>M. circinelloides</i>	0.01	0.09		
<i>M. rouxii</i>	0.01			
<i>M. plumbeus</i>		0.05		
<i>M. indicus</i>				
<i>Absidia corymbifera</i>	0.01			
<i>Cunninghamella bertholietiae</i>	0.01			
<i>C. albicans</i>				
<i>C. tropicalis</i>		0.02		
<i>C. krusei</i>				
<i>C. parasilosis</i>				
<i>C. glabrata</i>				
<i>Neosartorya pseudofischeri</i>		0.03		
<i>Blastomyces dermatitidis</i>	0.01			

TABLE 6-continued

Pseudallescheria and Sporothrix Probes				
Fungus	<i>P. boydii</i>	<i>P. marneffeii</i>	<i>P. notatum</i>	<i>Sporothrix schenckii</i>
<i>Apophysomyces elegans</i>	0.01			
Average Negative Controls	0.004 ± 0.002	0.013 ± 0.03	0.002 ± 0.019	0.001 ± 0.002

All of the references mentioned in this Specification are hereby incorporated by reference in their entirety.

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caacttttgt tgtataggat tattgggggc ctctcgatct gtatagatct tgaaatccct 180
gaaatttact aaggcctgaa cttgttttaa tgccctgaact tttttttaat ataaaggaaa 240
gctcttgtaa ttgacttga tggggcctcc caaataaatc tcttttaaat ttgatctgaa 300
atcaggcggg attaccgcgt gaacttaa 328

<210> SEQ ID NO 10
<211> LENGTH: 327
<212> TYPE: DNA
<213> ORGANISM: *Mucor plumbeus*

<400> SEQUENCE: 10

aaagtgcgat aactagtgtg aattgcatat tcagtgaatc atcgagtctt tgaacgcaac 60
ttgcgctcat tggattcca atgagcacgc ctgtttcagt atcaaaacaa accctctatc 120
caacttttgt tgtataggat tattgggggc ctctcgatct gtatagatct tgaaccctt 180
gaaatttact aaggcctgaa cttgttttaaat gcctgaactt ttttttaata taaaggaaag 240

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ctcttgtaat tgactttgat ggggcctccc aaataaatct tttttaaatt tgatctgaaa 300
tcaggtggga ttacccgctg aacttaa 327

<210> SEQ ID NO 11
<211> LENGTH: 322
<212> TYPE: DNA
<213> ORGANISM: *Mucor indicus*

<400> SEQUENCE: 11

aaagtgcgat aactagtgtg aattgcatat tcagtgaatc atcgagtctt tgaacgcatc 60
ttgcactcaa tggattcca ttgagtacgc ctgtttcagt atcaaaaaca acccttattc 120
aaaattcttt tttgaaatag atatgagtgt agcaacctta caagttgaga cattttaaat 180
aaagtcaggc catatcgtgg attgagtgcc gatacttttt taattttgaa aaggtaaagc 240
atgttgatgt ccgctttttg ggcctcccaa ataacttttt aaacttgatc tgaaatcagg 300
tgggattacc cgctgaactt aa 322

<210> SEQ ID NO 12
<211> LENGTH: 330
<212> TYPE: DNA
<213> ORGANISM: *Mucor circinelloides* f.

<400> SEQUENCE: 12

aaagtgcgat aactagtgtg aattgcatat tcagtgaatc atcgagtctt tgaacgcaac 60
ttgcgctcat tggattcca atgagcacgc ctgtttcagt atcaaaaca accctctatc 120
caacattttt gttgaaatag atgactgaga gtctcttgat ctattctgat ctcgaagctc 180
ttgaaatgta caaaggcctg atcttgtttg aatgcctgaa ctttttttta atataaagag 240
aagctcttgc ggtaaactgt gctggggcct cccaaataac acatctttaa atttgatctg 300
aaatcaggtg ggactaccg ctgaacttaa 330

<210> SEQ ID NO 13
<211> LENGTH: 333
<212> TYPE: DNA
<213> ORGANISM: *Rhizopus oryzae*

<400> SEQUENCE: 13

agtgcgataa ctagtgtgaa ttgcatattc agtgaatcat cgagtctttg aacgcagctt 60
gcactctatg gtttttctat agagtacgcc tgcttcagta tcatcaciaa cccacacata 120
acatttgttt atgtggtgat gggtcgcatc gctgttttat tacagtgagc acctaaaatg 180
tgtgtgattt tctgtctggc ttgctaggca ggaatattac gctggtctca ggatcttttt 240
ttttggttcg cccaggaagt aaagtacaag agtataatcc agtaactttc aaactatgat 300
ctgaagtcag gtgggattac ccgctgaact taa 333

<210> SEQ ID NO 14
<211> LENGTH: 333
<212> TYPE: DNA
<213> ORGANISM: *Rhizopus oryzae*

<400> SEQUENCE: 14

agtgcgataa ctagtgtgaa ttgcatattc agtgaatcat cgagtctttg aacgcagctt 60
gcactctatg gtttttctat agagtacgcc tgcttcagta tcatcaciaa cccacacata 120
acatttgttt atgtggtgat gggtcgcatc gctgttttat tacagtgagc acctaaaatg 180

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tgtgtgattt tctgtctggc ttgctaggca ggaatattac gctggtctca ggatcttttt 240
ctttggttcg cccaggaagt aaagtacaag agtataatcc agcaactttc aaactatgat 300
ctgaagtcag gtgggattac ccgctgaact taa 333
```

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<210> SEQ ID NO 15
<211> LENGTH: 348
<212> TYPE: DNA
<213> ORGANISM: Rhizopus microsporus
```

```
<400> SEQUENCE: 15
```

```
aaagtgcgat aactagtgtg aattgcatat tcgtgaatca tcgagtcttt gaacgcagct 60
tgcactctat ggatcttcta tagagtacgc ttgcttcagt atcataacca acccacacat 120
aaaatttatt ttatgtggtg atggacaagc tcggttaaat ttaattatta taccgattgt 180
ctaaaataca gcctctttgt aattttcatt aaattacgaa ctacctagcc atcgtgcttt 240
tttgggtcaa ccaaaaaaca tataatctag gggttctgct agccagcaga tattttaatg 300
atctttaact atgatctgaa gtcaagtggg actaccgct gaacttaa 348
```

```
<210> SEQ ID NO 16
<211> LENGTH: 349
<212> TYPE: DNA
<213> ORGANISM: Rhizopus microsporus
```

```
<400> SEQUENCE: 16
```

```
aaagtgcgat aactagtgtg aattgcatat tcgtgaatca tcgagtcttt gaacgcagct 60
tgcactctat ggatcttcta tagagtacgc ttgcttcagt atcataacca acccacacat 120
aaaatttatt ttatgtggtg atggacaagc tcggttaaat ttaattatta taccgattgt 180
ctaaaataca gcctctttgt aattttcatt aaattacgaa ctacctagcc atcgtgcttt 240
tttgggtcaa ccaaaaaaca tataatctag gggttctgct agccagcaaa tattttaatg 300
atctttaacc tatgatctga agtcaagtgg gactaccgct tgaacttaa 349
```

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<210> SEQ ID NO 17
<211> LENGTH: 361
<212> TYPE: DNA
<213> ORGANISM: Rhizopus circinans
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```
<400> SEQUENCE: 17
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```
aaattgcgat aactagtgtg aattgcattt tcagtgaatc atcgagtctt tgaacgcatc 60
ttgcgctctt gggattcttc cctagagcac acttgcttca gtatcataac aaaaccctca 120
cctaattttt tttttttta aaaaaaaaaat attagagtgg tattggggtc tctttgtaa 180
ttctttgtaa ttataaaagt acccttaaat gtcataaaca ggtagcttt agcttgcttt 240
taaagatctt cttagggtat cattaacttt cgtaaacttt taataggcct gtcacataat 300
tctaccctta aatttcttaa accttgatct gaagtcaagt gggagtaccg gctgaactta 360
a 361
```

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<210> SEQ ID NO 18
<211> LENGTH: 360
<212> TYPE: DNA
<213> ORGANISM: Rhizopus circinans
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```
<400> SEQUENCE: 18
```

```
aaattgcgat aactagtgtg aattgcattt tcagtgaatc atcgagtctt tgaacgcatc 60
ttgcgctctt gggattcttc cctagagcac acttgcttca gtatcataac aaaaccctca 120
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cctaataattt ttttttaaaa aaaaaaata ttagagtggg attggggctt ctttggtaat 180
tcttttgtaat tataaaagta cccttaaagc tcataaacag gttagcttta gcttgccctt 240
aaagatcttc ttagggatc attacttttc gtaaactctt aataggcctg tcacataatt 300
ctacccttaa atttcttaa ccttgatctg aagtcaagtg ggagtaccg ctgaacttaa 360

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<210> SEQ ID NO 19
<211> LENGTH: 486
<212> TYPE: DNA
<213> ORGANISM: Rhizopus stolonifer

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<400> SEQUENCE: 19

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aaagtgcgat aactagtgtg aattgcatat tcagtgaatc atcgagtctt tgaacgcaac 60
ttgactctta tggttttccg taaagtacgc ttgcttcagt atcataaaga ccccatcctg 120
attattattt ttttattaaa ataattaatt ttggagataa taaaaatgag gctctttctt 180
ttcttttttt tttttttaa aaaaaggggg ggaaaggggc ttttaaatg ggcaaattct 240
gggtttttta ctaaacctga actcccccca aaaattcaaa aaaaaaaaaa tgggttttac 300
caaatttttt tttttttct cctttttgtg tagttaatac tctattaaat ttatttactt 360
ggtattataa cgattatgca agaagggaga gaacaaagaa taatgaaaga gagtttttaa 420
ataaattctt ttttcattt ttcaatcaat gatctgaagt caagtgggat taccgctga 480
acttaa 486

```

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<210> SEQ ID NO 20
<211> LENGTH: 349
<212> TYPE: DNA
<213> ORGANISM: Rhizomucor pusillus

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<400> SEQUENCE: 20

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aaattgcgaa aagtaatgcy atctgcagcc tttgcaatc atcgaattct cgaacgcacc 60
ttgcaccctt tggttcatcc attgggtacg tctagttcag tatctttatt aaccctaaa 120
ggtttatttt ttgataaatc tttggatttg cgggtctgat ggattttcat ccgttcaagc 180
taccgaaca atttgtatgt tgttgacct tgatatttcc ttgaggcctt gcattggtat 240
ctaatttttt accagtgtgc ttcgagatga tcaagtataa aggtcaatca accacaaata 300
aatttcaact atggatctga acttagatgg gattaccgct tgaacttaa 349

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<210> SEQ ID NO 21
<211> LENGTH: 425
<212> TYPE: DNA
<213> ORGANISM: Absidia corymbifera

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```

<400> SEQUENCE: 21

```

```

aaagtgcgat aattattgcy acttgcatc atagcgaatc atcgagttct cgaacgcac 60
ttgagcctag tagtcaatct actaggcaca gttgtttcag tatctgcaac taccaatcag 120
ttcaacttgg ttctttgaac ctaagcgagc tggaaatggg cttgtgttga tggcattcag 180
ttgctgtcat ggccttaaat acatttagtc ctaggcaatt ggcttttagtc atttgccgga 240
tgtagactct agagtgcctg aggagcaacg acttggttag tgagttcata attccaagtc 300
aatcagtctc ttcttgaact aggtcttaat ctttatggac tagtgagagg atctaacttg 360
ggtcttctct taaaacaaac tcacatctag atctgaaatc aactgagatc acccgctgaa 420
cttaa 425

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<210> SEQ ID NO 22
<211> LENGTH: 399
<212> TYPE: DNA
<213> ORGANISM: Absidia corymbifera

<400> SEQUENCE: 22

aaagtgcgat aattattgcg acttgcatc atagtgaatc atcgagttct tgaacgcatc    60
ttgctgcctag tagtcaatct actaggcaca gttgtttcag tatctgcatc caccaatcaa    120
cttaaccttt tgtgttgagt tggaaactggg cttctagttg atggcattta gttgctgtca    180
tggccttaaa tcaatgtcct aggtgtaga acatctaaca ccggatggaa actttagagc    240
gctttaagag cagcttggtt agtgagttca ataattccaa gcattaagtc ttttaatgaa    300
ctagcttttc tatctatggg aactacttg gagaaatcca agtaaccttt aaactcccat    360
ttagatctga aatcaactga gaccaccgc tgaacttaa                                399

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<210> SEQ ID NO 23
<211> LENGTH: 359
<212> TYPE: DNA
<213> ORGANISM: Cunninghamella elegans

<400> SEQUENCE: 23

aaatcgcat atgtaatgtg actgcctata gtgaatcadc aaatctttga aacgcatctt    60
gcaccttatg gtattccata aggtacgtct gtttcagtac cactaataaa tctctctcta    120
tccttgatga tagaaaaaa aaaataaatt tttactgggc ccggggaatc cttttttttt    180
tttaataaaa aggaccaatt ttggcccaaa aaaaagggtt gaactttttt taccagatct    240
tgcacttagt aaaaacctag tcggctttaa tagattttta ttttctatta agtttatagc    300
cattcttata ttttttaaaa tcttggcctg aaatcagatg ggataccgcg tgaacttaa    359

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<210> SEQ ID NO 24
<211> LENGTH: 346
<212> TYPE: DNA
<213> ORGANISM: Pseudallescheria boydii

<400> SEQUENCE: 24

aaatgcgata agtaatgtaa attgcaaaat tcagtgaatc atcgaatctt tgaaacgcac    60
attgccccg gcagtaatct gccgggcatg cctgtccgag cgtcatttca accctcgaac    120
ctccgtttcc ttagggaagc ctagggtcgg tgttggggcg ctacggcaag tcctcgcaac    180
ccccgtaggc cctgaaatac agtggcggtc ccgccgggt tgccttctgc gtagtaagtc    240
tcttttgcaa gctcgattg ggtcccggcg gaggcctgcc gtcaaaccac ctaacaactc    300
cagatggttt gacctcggat caggtagggt taccgctga acttaa                                346

```

```

<210> SEQ ID NO 25
<211> LENGTH: 346
<212> TYPE: DNA
<213> ORGANISM: Pseudallescheria boydii

<400> SEQUENCE: 25

gaaatgcgat aagtaatgtg aattgcagaa ttcagtgaat catcgaatct ttgaaacgca    60
cattgcccc ggagtaatc tgccgggcat gcctgtccga gcgtcatttc aaccctcgaac    120
cctccgtttc ctgaggaag ctgagggtcg gtgttggggc gctacggcaa gtcttcgcaa    180
ccctccgtag gccctgaaat acagtggcgg tcccgccgcg gttgccttct gcgtagaagt    240
ctcttttgca agctcgatt gggccccggc ggaggcctgc cgtcaaacca cctataactc    300

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caaatggttt gacctcggat caggtagggt taccogctga acttaa 346

<210> SEQ ID NO 26
 <211> LENGTH: 344
 <212> TYPE: DNA
 <213> ORGANISM: *Scedosporium apiospermum*

<400> SEQUENCE: 26

gaaatgcgat aagtaatgtg aattgcagaa ttcagtgaat catcgaatct ttgaacgcac 60
 attgcgcccc gcagtaatct gccgggcatg cctgtccgag cgtcatttca accctcgaac 120
 ctccgtttcc tcaggaagc tcagggtcgg tgttggggcg ctacggcgag tcttcgcgac 180
 cctccgtagg ccctgaaata cagtggcggg cccgccgagg ttgccttctg cgtagtaagt 240
 ctcttttgca agctcgcatt gggccccggc ggaggcctgc cgtcaaacca cctataactc 300
 cagatggttt gacctcggat caggtaggta cccgctgaac ttaa 344

<210> SEQ ID NO 27
 <211> LENGTH: 343
 <212> TYPE: DNA
 <213> ORGANISM: *Scedosporium apiospermum*

<400> SEQUENCE: 27

aaatgcgata agtaatgtga attgcagaat tcagtgaatc atcgaatctt tgaacgcaca 60
 ttgcgccccg cagtaatctg ccgggcatgc ctgtccgagc gtcatttcaa ccctcgaacc 120
 tccgtttcct caggaagct cagggtcggg gttggggcgc tacggcgagt cttcgcgacc 180
 ctccgtaggc cctgaaatac agtggcgggc ccgccgagg tgccttctgc gtagtaagtc 240
 tcttttgcaa gctcgcattg ggtccccggc gaggcctgcc gtcaaaccac ctataactcc 300
 agatggtttg acctcggatc aggtaggtag ccgctgaact taa 343

<210> SEQ ID NO 28
 <211> LENGTH: 309
 <212> TYPE: DNA
 <213> ORGANISM: *Penicillium notatum*

<400> SEQUENCE: 28

aaatgcgata cgtaatgtga attgcaaatt cagtgaatca tcgagtcttt gaacgcacat 60
 tgcgccccct ggtattccgg ggggcatgcc tgtccgagcg tcattgctgc cctcaagcac 120
 ggcttgtgtg ttgggccccg tcctccgata ccgggggagc ggcccgaag gcagcggcgg 180
 caccgcgtcc ggtcctcagc cgtatggggc tttgtcacc gctctgtagg cccggccggc 240
 gcttgccgat caaccctaat ttttatccag gttgacctcg gatcaggtag ggataccgcg 300
 tgaacttaa 309

<210> SEQ ID NO 29
 <211> LENGTH: 336
 <212> TYPE: DNA
 <213> ORGANISM: *Sporothrix schenckii*

<400> SEQUENCE: 29

gaaatgcgat actaatgtga attgcagaat tcagcgaacc atcgaatctt tgaacgcaca 60
 ttgcgccccg cagcattctg gcgggcatgc ctgtccgagc gtcatttccc ccctcacgcg 120
 ccccgttgcg cgctgggtgt ggggcgccct ccgctggcg gggggcccc gaaagcgagt 180
 ggcgggccct gtggaaggct ccgagcgcag taccgaacgc atgttctccc ctgcctcgg 240

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aggccccca ggcgcctgc cggtgaaaac ggcgatgacg cgcagctctt tttaacaagg 300
 tgacctcgga tcaggtgagg ataccgctg acttaa 336

<210> SEQ ID NO 30
 <211> LENGTH: 18
 <212> TYPE: DNA
 <213> ORGANISM: *Aspergillus flavus*

<400> SEQUENCE: 30
 gcaaatcaat ctttttcc 18

<210> SEQ ID NO 31
 <211> LENGTH: 18
 <212> TYPE: DNA
 <213> ORGANISM: *Aspergillus fumigatus*

<400> SEQUENCE: 31
 gaacgcaaat caatcttt 18

<210> SEQ ID NO 32
 <211> LENGTH: 18
 <212> TYPE: DNA
 <213> ORGANISM: *Aspergillus fumigatus*

<400> SEQUENCE: 32
 ccgacacca tctttatt 18

<210> SEQ ID NO 33
 <211> LENGTH: 18
 <212> TYPE: DNA
 <213> ORGANISM: *Aspergillus niger*

<400> SEQUENCE: 33
 gacgttatcc aaccattt 18

<210> SEQ ID NO 34
 <211> LENGTH: 18
 <212> TYPE: DNA
 <213> ORGANISM: *Aspergillus terreus*

<400> SEQUENCE: 34
 gcatttattt gcaacttg 18

<210> SEQ ID NO 35
 <211> LENGTH: 18
 <212> TYPE: DNA
 <213> ORGANISM: *Aspergillus nidulans*

<400> SEQUENCE: 35
 ggcgtctcca accttatc 18

<210> SEQ ID NO 36
 <211> LENGTH: 18
 <212> TYPE: DNA
 <213> ORGANISM: *Mucor rouxii*

<400> SEQUENCE: 36
 gaataggaat actgagag 18

<210> SEQ ID NO 37
 <211> LENGTH: 15
 <212> TYPE: DNA
 <213> ORGANISM: *Mucor indicus*

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<400> SEQUENCE: 37
gaaacccttg aaatt 15

<210> SEQ ID NO 38
<211> LENGTH: 18
<212> TYPE: DNA
<213> ORGANISM: *Mucor indicus*

<400> SEQUENCE: 38
cgtggattga gtgccgat 18

<210> SEQ ID NO 39
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: *Mucor circinelloides* f.

<400> SEQUENCE: 39
aacatttttg tgaataggat g 21

<210> SEQ ID NO 40
<211> LENGTH: 15
<212> TYPE: DNA
<213> ORGANISM: *Mucor racemosus*

<400> SEQUENCE: 40
gaaatccctg aaatt 15

<210> SEQ ID NO 41
<211> LENGTH: 18
<212> TYPE: DNA
<213> ORGANISM: *Rhizopus oryzae*

<400> SEQUENCE: 41
gagtataatc cagyaact 18

<210> SEQ ID NO 42
<211> LENGTH: 18
<212> TYPE: DNA
<213> ORGANISM: *Rhizopus circinans*

<400> SEQUENCE: 42
cttagggtat cactactt 18

<210> SEQ ID NO 43
<211> LENGTH: 18
<212> TYPE: DNA
<213> ORGANISM: *Rhizomucor pusillus*

<400> SEQUENCE: 43
tccttgaggg cttgcatt 18

<210> SEQ ID NO 44
<211> LENGTH: 18
<212> TYPE: DNA
<213> ORGANISM: *Rhizopus stolonifer*

<400> SEQUENCE: 44
cttggtatta taacgatt 18

<210> SEQ ID NO 45
<211> LENGTH: 18
<212> TYPE: DNA

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<213> ORGANISM: *Pseudallescheria boydii*

<400> SEQUENCE: 45

aagtctcttt tgcaagct 18

<210> SEQ ID NO 46

<211> LENGTH: 18

<212> TYPE: DNA

<213> ORGANISM: *Penicillium notatum*

<400> SEQUENCE: 46

gatcaaccca aattttta 18

<210> SEQ ID NO 47

<211> LENGTH: 18

<212> TYPE: DNA

<213> ORGANISM: *Penicillium marneffeii*

<400> SEQUENCE: 47

gggttggtca ccaccata 18

<210> SEQ ID NO 48

<211> LENGTH: 18

<212> TYPE: DNA

<213> ORGANISM: *Penicillium marneffeii*

<400> SEQUENCE: 48

tggtcaccac catattta 18

<210> SEQ ID NO 49

<211> LENGTH: 18

<212> TYPE: DNA

<213> ORGANISM: *Fusarium moniliforme*

<400> SEQUENCE: 49

tctagtgacg gtctcgct 18

<210> SEQ ID NO 50

<211> LENGTH: 18

<212> TYPE: DNA

<213> ORGANISM: *Fusarium oxysporum*

<400> SEQUENCE: 50

cgttaattcg cgttcctc 18

<210> SEQ ID NO 51

<211> LENGTH: 21

<212> TYPE: DNA

<213> ORGANISM: *Fusarium solani*

<400> SEQUENCE: 51

ctaacacctc gcaactggag a 21

<210> SEQ ID NO 52

<211> LENGTH: 18

<212> TYPE: DNA

<213> ORGANISM: *Cunninghamella elegans*

<400> SEQUENCE: 52

tagtcggctt taatagat 18

<210> SEQ ID NO 53

<211> LENGTH: 18

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<212> TYPE: DNA
 <213> ORGANISM: *Cunninghamella elegans*

 <400> SEQUENCE: 53
 tattaagttt atagccat 18

<210> SEQ ID NO 54
 <211> LENGTH: 18
 <212> TYPE: DNA
 <213> ORGANISM: *Cunninghamella elegans*

 <400> SEQUENCE: 54
 taagtttata gccattct 18

<210> SEQ ID NO 55
 <211> LENGTH: 18
 <212> TYPE: DNA
 <213> ORGANISM: *Absidia corymbifera*

 <400> SEQUENCE: 55
 gttgctgtca tggcctta 18

<210> SEQ ID NO 56
 <211> LENGTH: 18
 <212> TYPE: DNA
 <213> ORGANISM: *Sporothrix schenckii*

 <400> SEQUENCE: 56
 gacgcgcagc tcttttta 18

<210> SEQ ID NO 57
 <211> LENGTH: 18
 <212> TYPE: DNA
 <213> ORGANISM: *Rhizopus microsporus*

 <400> SEQUENCE: 57
 catataatct aggggttc 18

<210> SEQ ID NO 58
 <211> LENGTH: 18
 <212> TYPE: DNA
 <213> ORGANISM: *Aspergillus sp.*

 <400> SEQUENCE: 58
 cctcgagcgt atggggct 18

<210> SEQ ID NO 59
 <211> LENGTH: 18
 <212> TYPE: DNA
 <213> ORGANISM: *Fusarium sp.*

 <400> SEQUENCE: 59
 cccaacttct gaatgttg 18

<210> SEQ ID NO 60
 <211> LENGTH: 18
 <212> TYPE: DNA
 <213> ORGANISM: *Mucor sp.*

 <400> SEQUENCE: 60
 mtggggcctc ccaaataa 18

<210> SEQ ID NO 61

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<211> LENGTH: 21
 <212> TYPE: DNA
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Description of Artificial Sequence: B-58 biotin probe

<400> SEQUENCE: 61

gaatcatcga rtctttgaac g

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We claim:

1. An isolated nucleic acid probe that consists essentially of 10 to 50 consecutive nucleotides for species-specific identification of *Aspergillus*, wherein the probe selectively hybridizes under stringent conditions to the internal transcribed spacer 2 nucleic acid sequence of one of *Aspergillus flavus* (SEQ ID NO:1), *Aspergillus fumigatus* (SEQ ID NO:2), *Aspergillus niger* (SEQ ID NO:3), *Aspergillus terreus* (SEQ ID NO:4), or *Aspergillus nidulans* (SEQ ID NO:5), but does not selectively hybridize under stringent conditions to the internal transcribed spacer 2 region of any other *Aspergillus* species, nor does it hybridize to the internal transcribed spacer 2 nucleic acid sequence of *Fusarium solani* (SEQ ID NO:6), *Fusarium moniliforme* (SEQ ID NO:7), *Mucor rouxii* (SEQ ID NO:8), *Mucor racemosus* (SEQ ID NO:9), *Mucor plumbeus* (SEQ ID NO:10), *Mucor indicus* (SEQ ID NO:11), *Mucor circinilloides f. circinelloides* (SEQ ID NO:12), *Rhizopus oryzae* (SEQ ID NO:13 and NO:14), *Rhizopus microsporus* (SEQ ID NO:15 and 16), *Rhizopus circinans* (SEQ ID NO:17 and 18), *Rhizopus stolonifer* (SEQ ID NO:19), *Rhizomucor pusillus* (SEQ ID NO:20), *Absidia corymbifera* (SEQ ID NO:21 and 22), *Cunninghamella elegans* (SEQ ID NO:23), *Pseudallescheria boydii* (teleomorph of *Scedosporium apiospermum*) (SEQ ID NO:24, 25, 26, and 27), *Penicillium notatum* (SEQ ID NO:28), or *Sporothrix schenckii* (SEQ ID NO:29).

2. The isolated nucleic acid probe of claim 1 wherein the probe selectively hybridizes with an *Aspergillus flavus* nucleic acid of SEQ ID NO:1, or a complementary sequence thereof.

3. The isolated nucleic acid probe of claim 1 wherein the probe selectively hybridizes with an *Aspergillus fumigatus* nucleic acid of SEQ ID NO:2, or a complementary sequence thereof.

4. The isolated nucleic acid probe of claim 1 wherein the probe selectively hybridizes with an *Aspergillus niger* nucleic acid of SEQ ID NO:3, or a complementary sequence thereof.

5. The isolated nucleic acid probe of claim 1 wherein the probe selectively hybridizes with an *Aspergillus terreus* nucleic acid of SEQ ID NO:4, or a complementary sequence thereof.

6. The isolated nucleic acid probe of claim 1 wherein the probe selectively hybridizes with an *Aspergillus nidulans* nucleic acid of SEQ ID NO:5, or a complementary sequence thereof.

7. A method of detecting a species of *Aspergillus flavus* (SEQ ID NO:1), *Aspergillus fumigatus* (SEQ ID NO:2), *Aspergillus niger* (SEQ ID NO:3), *Aspergillus terreus* (SEQ ID NO:4), or *Aspergillus nidulans* (SEQ ID NO:5) in a sample comprising

contacting the sample with a nucleic acid probe consisting essentially of 10 to 50 consecutive nucleotides that

selectively hybridizes with a nucleic acid having a sequence as set forth as SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, or SEQ ID NO:5, or a complementary sequence thereof;

wherein hybridization of the nucleic acid probe with the sample indicates the detection of the *Aspergillus* species in the sample.

8. The method of claim 7, wherein the probe selectively hybridizes with an *Aspergillus flavus* nucleic acid of SEQ ID NO:1, or a complementary sequence thereof.

9. The method of claim 7, wherein the probe selectively hybridizes with an *Aspergillus fumigatus* nucleic acid of SEQ ID NO:2, or a complementary sequence thereof.

10. The method of claim 7, wherein the probe selectively hybridizes with an *Aspergillus niger* nucleic acid of SEQ ID NO:3, or a complementary sequence thereof.

11. The method of claim 7, wherein the probe selectively hybridizes with an *Aspergillus terreus* nucleic acid of SEQ ID NO:4, or a complementary sequence thereof.

12. The method of claim 7, wherein the probe selectively hybridizes with an *Aspergillus nidulans* nucleic acid of SEQ ID NO:5, or a complementary sequence thereof.

13. An isolated nucleic acid probe for identifying a filamentous fungus wherein the probe consists essentially of a nucleic acid having a sequence as set forth as SEQ ID NO:61, or a complementary sequence thereof, respectively.

14. The isolated nucleic acid probe of claim 1, wherein the probe consists essentially of a nucleotide sequence having a sequence as set forth as SEQ ID NO:30, SEQ ID NO:31, SEQ ID NO:32, SEQ ID NO:33, SEQ ID NO:34, or SEQ ID NO:35.

15. The isolated nucleic acid probe of claim 1, wherein the probe consists essentially of the nucleotide sequence set forth as SEQ ID NO:30.

16. The isolated nucleic acid probe of claim 1, wherein the probe consists essentially of the nucleotide sequence set forth as SEQ ID NO:31.

17. The isolated nucleic acid probe of claim 1, wherein the probe consists essentially of the nucleotide sequence set forth as SEQ ID NO:32.

18. The isolated nucleic acid probe of claim 1, wherein the probe consists essentially of the nucleotide sequence set forth as SEQ ID NO:33.

19. The isolated nucleic acid probe of claim 1, wherein the probe consists essentially of the nucleotide sequence set forth as SEQ ID NO:34.

20. The isolated nucleic acid probe of claim 1, wherein the probe consists essentially of the nucleotide sequence set forth as SEQ ID NO:35.

21. The method of claim 7, wherein the probe consists essentially of a nucleotide sequence as set forth as SEQ ID NO:30, SEQ ID NO:31, SEQ ID NO:32, SEQ ID NO:33, and SEQ ID NO:34.

22. The method of claim 7, wherein the probe consists essentially of the nucleotide sequence set forth as SEQ ID NO:30.

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23. The method of claim 7, wherein the probe consists essentially of the nucleotide sequence set forth as SEQ ID NO:31.

24. The method of claim 7, wherein the probe consists essentially of the nucleotide sequence set forth as SEQ ID NO:32.

25. The method of claim 7, wherein the probe consists essentially of the nucleotide sequence set forth as SEQ ID NO:33.

26. The method of claim 7, wherein the probe consists essentially of the nucleotide sequence set forth as SEQ ID NO:34.

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27. The isolated nucleic acid probe of claim 1, wherein the probe consists essentially of the nucleotide sequence set forth as SEQ ID NO:35.

28. An isolated nucleic acid comprising a sequence as set forth as SEQ ID NO:1 or SEQ ID NO:2.

29. An isolated nucleic acid consisting essentially of a sequence as set forth as SEQ ID NO:3, SEQ ID NO:4 or SEQ ID NO:5.

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